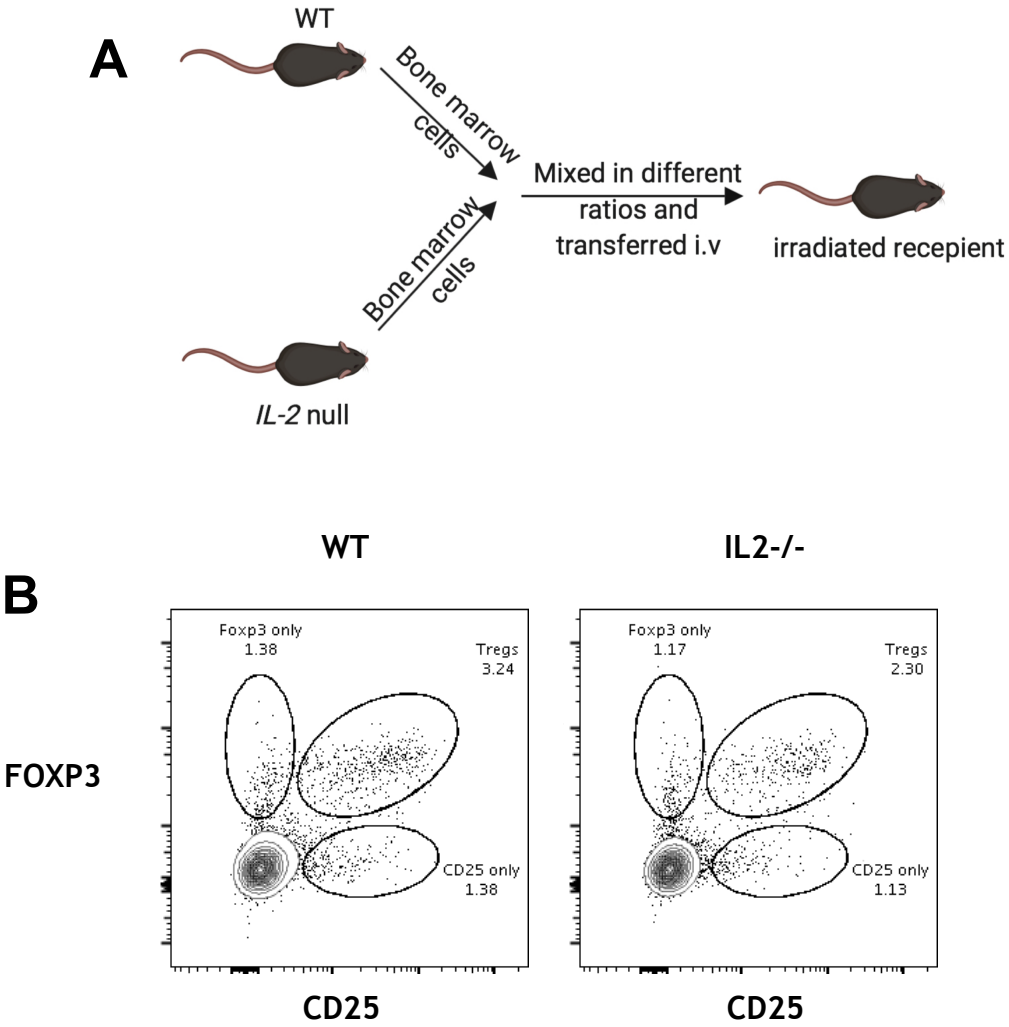
Supplementary Figures

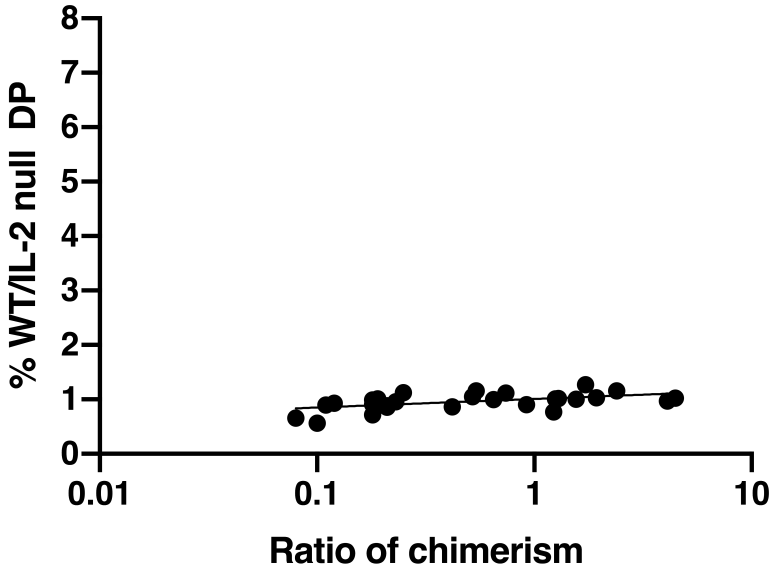
A role for cell-autocrine interleukin-2 in regulatory T cell homeostasis (Chawla et al)

Figure S1



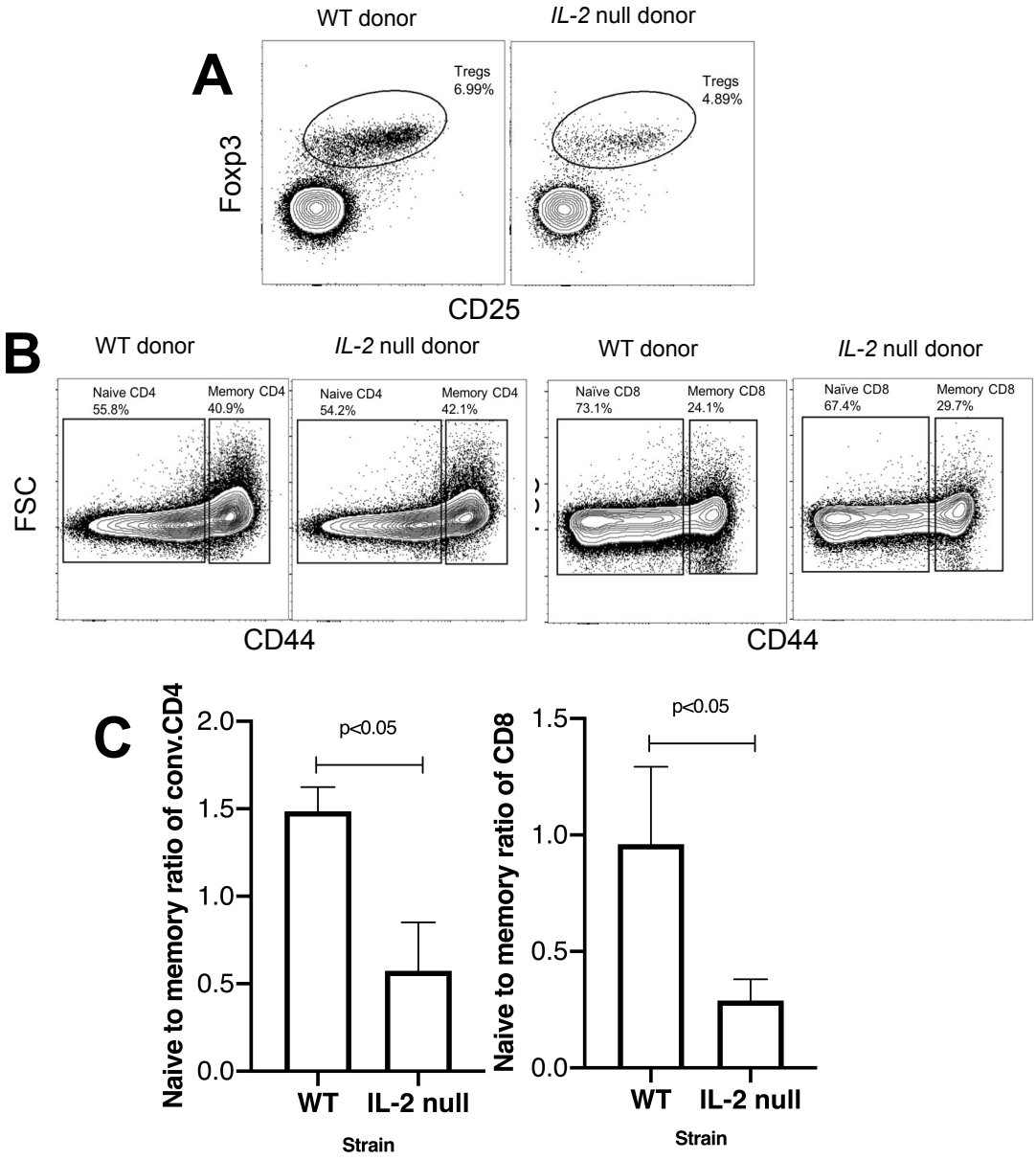
Thymocytes from mixed bone marrow chimeras were isolated and analysed by flo cytometry. Bone marrow chimeras were made with a wide range of WT:IL2-/- bone marrow cells.
(A) schematic representation of strategy followed for making mixed bone marrow chimeras.
(B) Representative flow cytometric plots for immature and mature Tregs in thymocytes of chimeras gated on either WT (CD45.1) or IL2-/- (CD45.2) genotypes. Tregs were gated as CD25+FOXP3+ CD4+CD8- and Treg precursors were gated as CD25+FOXP3- CD4+CD8- or CD25-FOXP3+ CD4+CD8-.

Figure S2



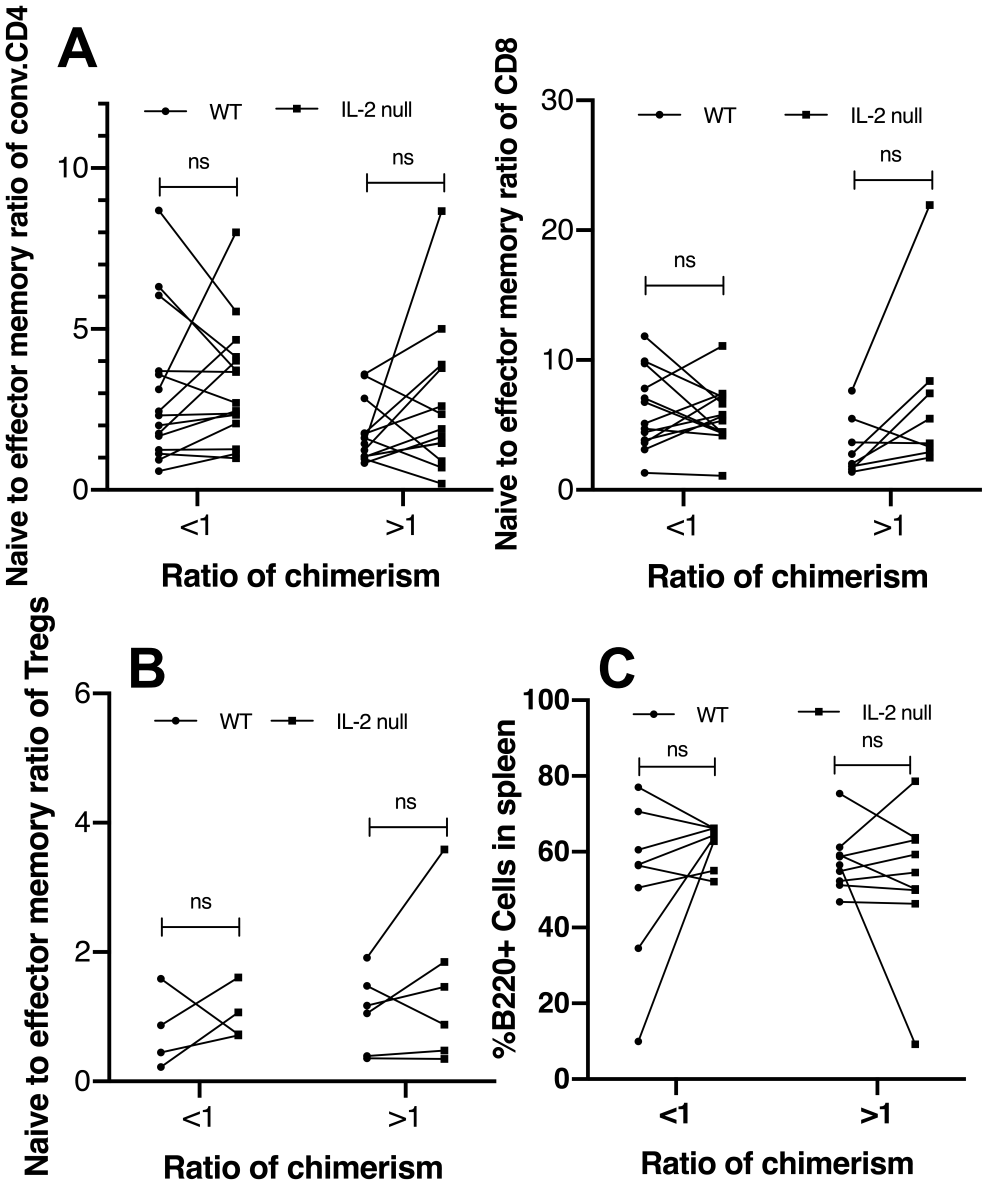
Thymocytes from mixed bone marrow chimeras were isolated and analysed by flow cytometry. Bone marrow chimeras were made with a wide range of WT:IL2-/- bone marrow cells. WT:IL2-/- donor genotype ratios for DP cells (CD4+CD8+) were calculated in each individual recipient mouse and plotted versus output chimerism in each recipient mouse.

Figure S3



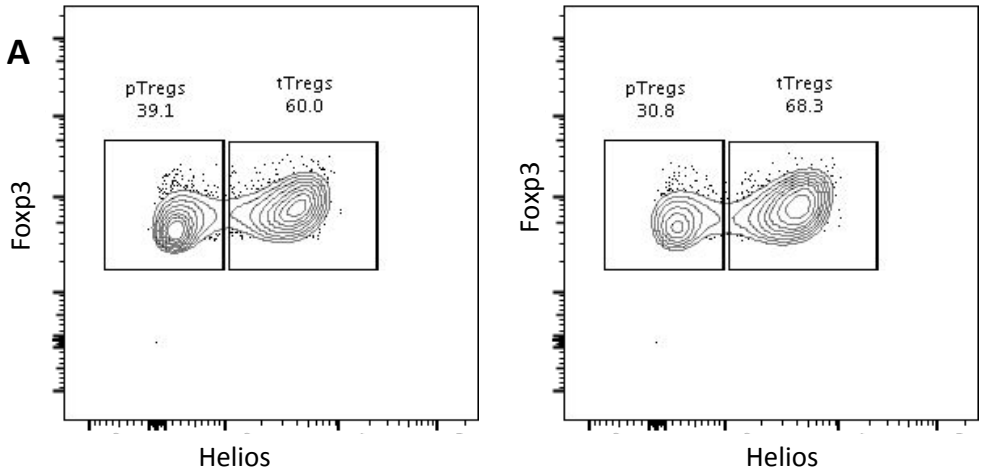
Splenic cells from WT:IL2-/- mixed bone marrow chimeras were isolated and analysed by flow cytometry.
(A) Representative flow cytometric plots of splenic Tregs gated as CD4+CD25+FOXP3+, gated on either WT (CD45.1) or IL2-/- (CD45.2).
(B) Representative flow cytometric plots of peripheral naïve:memory CD4 and CD8 T cells in mixed bone marrow chimeras gated on either CD45.1 (WT) or CD45.2 (IL2-/-) donors. Naïve cells were gated as CD4+CD44loCD25- or CD8+CD44lo, while memory T cells were gated as CD4+CD44hi CD25- or CD8+CD44hi.
(C) Quantification of naïve:memory ratios in parental strains. Naïve cells were gated as shown in (B) above. Student's t test was used to calculate p values.

Figure S4



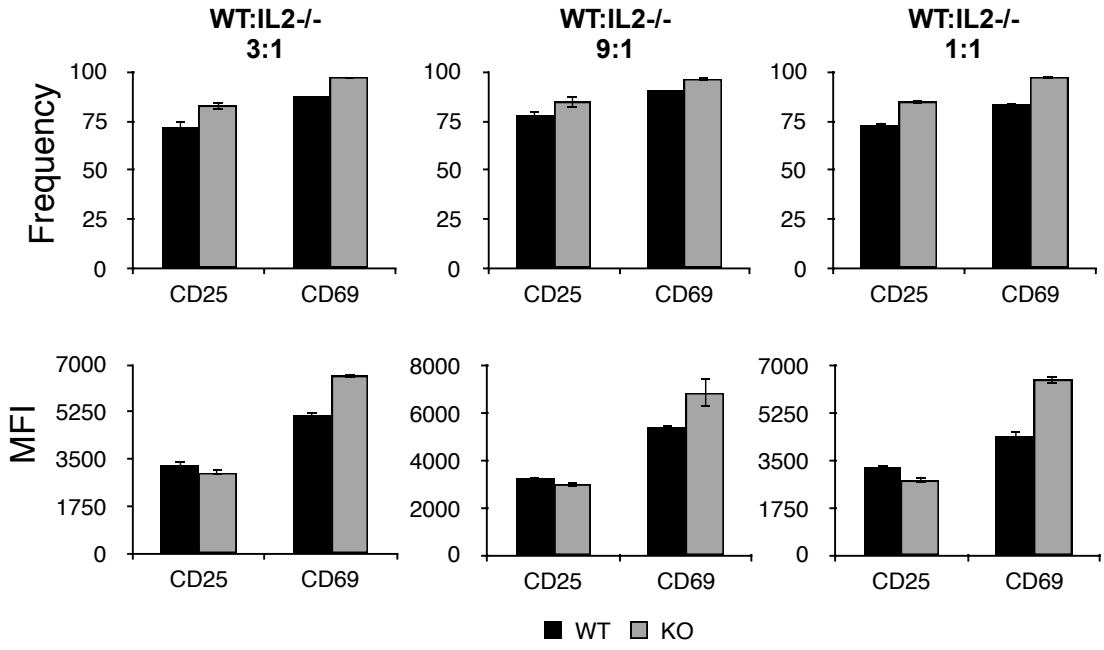
Splenic cells from mixed bone marrow chimeras were isolated and analysed by flow cytometry. For further analysis, bone marrow chimeras were divided into two groups, one with a chimerism ratio of <1 (range: 0.1-0.9), and the second with a ratio of >1 (range: 1.1-48). For each chimera group, paired data are shown for WT versus IL2-/- donor genotypes in each recipient. Quantification of various subsets in mixed bone marrow chimeras gated on either CD45.1 (WT) or CD45.2 (IL2-/-) donors is shown. Student's t-test was used to quantify p values. ns: p>0.05.
(A) Quantification of naïve:effector memory ratios in CD4 or CD8 T cells as shown in chimeras in eitherWTIL2-/- donors. Naïve cells were gated as CD4+CD44loCD62LhiCD25- or CD8+CD44loCD62Lhi, while memory T cells were gated as CD4+CD44hiCD62Llo CD25- or CD8+CD44hiCD62Llo.
(B) Quantification of naïve:memory ratios in Treg cells in chimeras in either WT or IL2-/- donors. Naïve Treg cells were gated as CD4+CD44loCD62LhiCD25+FOXP3+, while memory Treg cells were gated as CD4+CD44hiCD62LloCD25+FOXP3+.
(C) Quantification of splenic B cell frequencies in chimeras in either WT or IL2-/- donors.

Figure S5



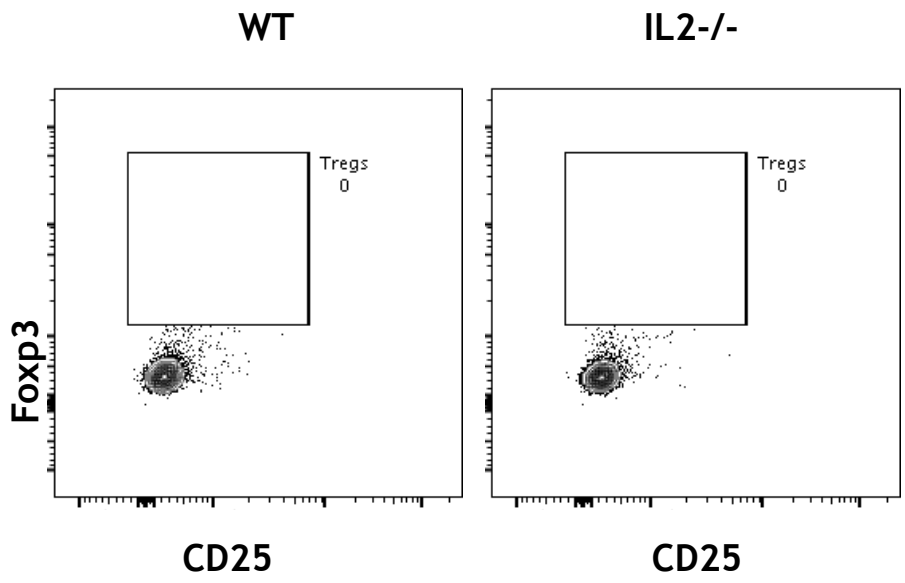
Splenic cells from WT:IL2-/- mixed bone marrow chimeras were isolated and analysed by flow cytometry.
(A) Representative flow cytometric plots of Tregs gated as CD4+CD25+FOXP3+, gated on either WT (CD45.1) or IL2-/- (CD45.2), stained for FOXP3 versus Helios to identify tTreg and pTreg cell subsets.

Figure S6



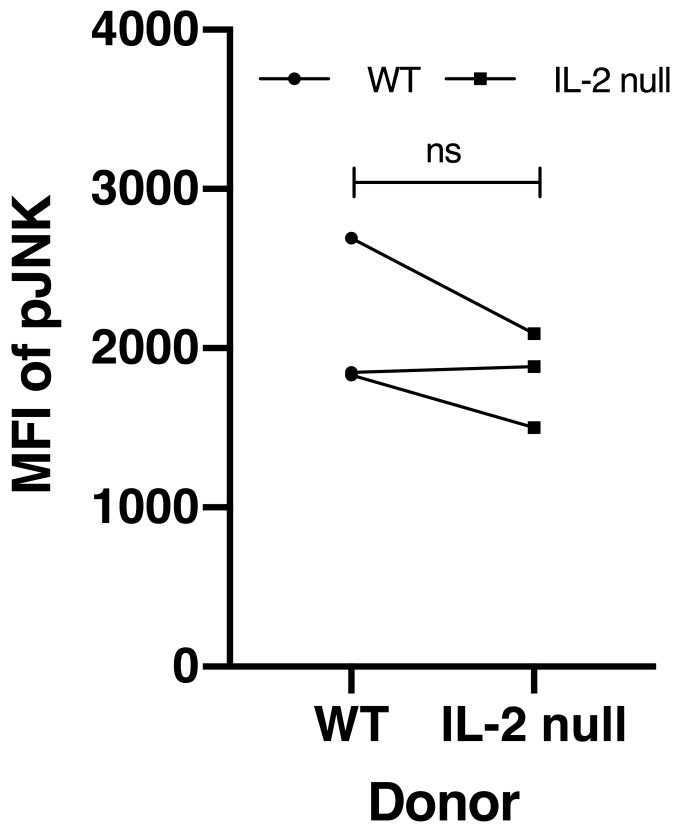
Naïve CD4 T cells (CD4+CD25-CD44lo) cells from both WT and IL2-/- (KO) donor genotypes were isolated from mixed bone marrow chimeras and mixed in different ratios as shown, and stimulated with plate-coated anti-CD3+anti-CD28 and TGF-beta in the presence of exogenous IL2. Cells were stained for CD69 or CD25 at 24 h post-activation and both frequencies and intensities (MFI) were quantified (n=3). Data are representative of two independent experiments.

Figure S7



Naïve CD4 T cells (CD4+CD25-CD44lo) from both WT and IL2-/- donor partners were isolated from mixed bone marrow chimeras for transfer for in vivo pTreg generation. They were tested for FOXP3 expression as shown above, and exhibited no residual pre-existing Treg contamination.

Figure S8



Tregs (CD4+CD25+FOXP3+) from spleen of mixed bone chimeras were analysed by flow cytometry for mean fluorescent intensity of phospho-JNK as shown.